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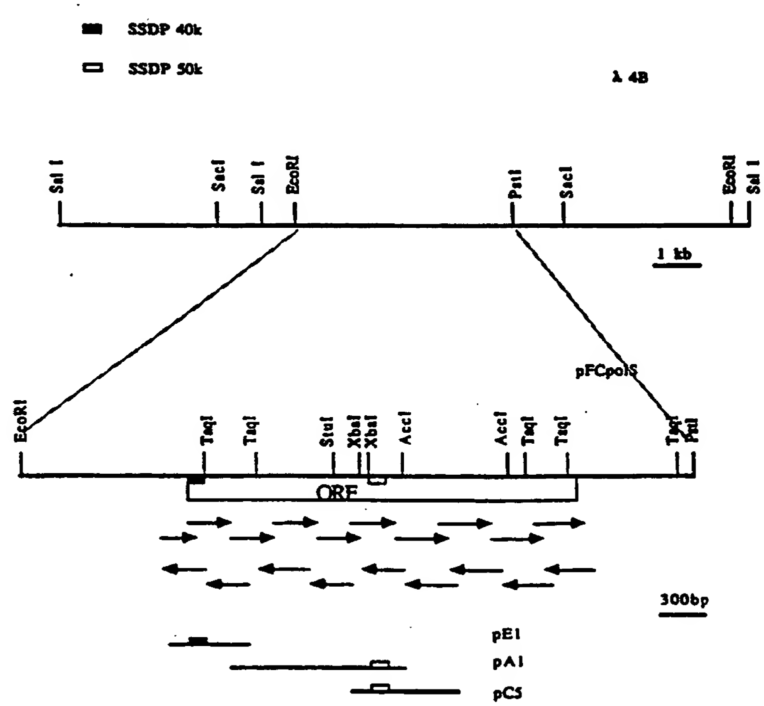
(54) Title: NUCLEOTIDE SEQUENCES CODING FOR A THERMOSTABLE DNA POLYMERASE, DNA POLYMERASE AND USES THEREOF



(57) Abstract

Nucleotide sequences coding a polypeptide or fragments thereof having a thermostable and thermophilic DNA polymerase activity, preferably derived from DNA of bacteria of *Sulfolobus genus*, DNA polymerase and uses thereof.

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Nucleotide Sequences Coding for a Thermostable DNA Polymerase, DNA Polymerase and Uses Thereof

Specification

The present invention concerns the isolation and the identification of sequences coding a DNA polymerase from bacteria belonging to the *Archaea* domain (Woese C.R. et al. 1990, Proc. Natl. Acad.Sci. USA 87, 4576-4579), to the protein coded by said sequence and to uses thereof.

DNA polymerases are enzymes responsible of the duplication of genomic DNA and, therefore, of the inheritance of the genetic material. Sequences coding DNA polymerase from bacteria belonging to the *Archaea* domain are not known in the prior art. Such bacteria are adapted to grow at high temperatures, and are evolutionary far from *Eubacteria*.

DNA polymerases may be classified in two classes (Ito, J., and Braithwaite, D.K. (1991) Nucleic Acids Res. 19, 4045-4057). Class A comprises dideoxynucleotide inhibition sensitive and aphidicolin resistant enzymes, as pol I from *E. coli* (Joyce, C.M., Kelley, W.S., and Grindley, N.D. F. (1982) J. Biol. Chem. 257, 1958-1964); class B is more heterogeneous, comprising aphidicolin sensitive and partially dideoxynucleotide inhibition resistant enzymes.

The authors of the instant invention have demonstrated that DNA polymerase extracted from bacteria of thermostable and thermofilic *Sulfolobus solfataricus* species has a molecular weight of around 100 kDa, by means of gel filtration chromatoghraphy and of glycerol gradient centrifugation. An electrophoresis in denaturing

conditions on polyacrylammide gel shows, other than the 100 kDa protein, two major bands, respectively of 50 e 40 kDa. These bands represent proteolytic cleavage fragments of the 100 kDa protein, being able to react with antisera raised against the native 100 kDa protein. Moreover the 50 kDa fragment keeps a DNA polymerase activity (Karawya, E., Swack, J.A., and Wilson, S.H. (1983) Anal. Biochem. 135, 318-325).

The authors of the present invention have isolated and sequenced the gene coding the DNA polymerase from *S. solfataricus*, and have deduced the aminoacid sequence of the protein. Upon insertion into procaryotic or eucaryotic expression vectors and transformation of suitable hosts, the gene makes possible the production through recombinant DNA techniques of the DNA polymerase enzyme.

According to the invention the term "thermofilic" refers to enzymes with a peak of activity at temperatures comprised between 50°C and 85°C, preferably 75°C, when a substrate of DNA from activated calf thymus is used; the term "thermostable" refers to the fact that the enzyme keeps 100% of activity after incubation for 40 min at 75°C.

It is an object of the invention a nucleic acid of natural, recombinant or synthetic origin, comprising a nucleotide sequence coding a polypeptide or fragments thereof having a thermostable and thermofilic DNA polymerase activity. Preferably said nucleotide sequence is derived from DNA of bacteria of the *Archaea* domain, preferably of the *Sulfolobus* genus, more preferably of the *S. solfataricus* species.

In a preferred embodiment said polypeptide or fragments thereof have also a 3'-5' exonuclease activity.

Preferably said nucleotide sequence codes the polypeptide having the aminoacid sequence of SEQ ID N2, or fragments thereof, alternatively deleted or substituted for one or more aminoacids, so that said DNA polymerase activity is maintained.

Further object of the invention is a nucleic acid comprised in the sequence of SEQ ID N1 characterized in that from nucleotide 1 to nucleotide 197 is a non coding sequence, from nucleotide 198 to nucleotide 2843 coding a polypeptide with a thermostable and thermofilic DNA polymerase activity and from nucleotide 2844 to nucleotide 3112 is a non coding sequence. Alternatively said nucleotide sequence lacks or is substituted of one or more nucleotides so that said DNA polymerase activity is maintained.

Another object of the invention are nucleotide sequences able to hybridize at medium stringency to nucleotide sequences of the invention, preferably said sequences are complementary to the sequences of the invention.

It is another object of the invention a polypeptide with a thermostable and thermofilic DNA polymerase activity, preferably produced through recombinant DNA techniques by nucleotide sequences according to the invention, preferably by the nucleotide sequence comprised in SEQ ID N1.

According to the invention said polypeptide has a sequence comprised in SEQ ID N2.

It is a further object of the invention recombinant cloning or expression vectors, having a plasmid or viral derivation, comprising the

nucleotide sequences of the invention, preferably said vector is the plasmid pFCpolS (DSM N.7091).

Another object are cells transformed with said vectors.

The invention will be described in the following examples, with reference to the following figures:

figure 1 which represents a restriction map of the coding region of the DNA polymerase gene of *S. solfataricus;*

figures 2a and 2b which represent a sequence analysis of DNA polymerase sequences from different organisms.

Example 1 <u>Partial aminoacid sequence of DNA polymerase from *S. solfataricus*</u>

30 μg of DNA polymerase purified from *S. solfataricus*, as described in Rossi M. et al. 1986, System. Appl. Microbiol. 7, 337-341, is loaded on a 10% polyacrylammide gel in denaturing conditions. The gel is then electro-transferred on a PVDF membrane (Problott, Applied Biosystems), as described in Matsudaira, P. (1987) J. Biol. Chem. 262, 10035-10038. The membrane is stained with Coomassie Brilliant Blue R-250. Three protein bands of 100, 50 e 40 kDa are cutted and loaded directly on a gas-phase aminoacid sequencer (M. 470 A, Applied Biosystems), with an analyzer PTH 120 A. N-terminal sequences of 50 e 40 kDa peptides are:

50 kDa GYKGAVVIDP

40 kDa SAPVEEKKVVR

Example 2 <u>Isolation and sequence of the DNA polymerase gene of *S. solfataricus*</u>

By using aminoacid sequences the following degenerated oligonucleotides are sinthesized:

29-mer SSDP50K corresponding to the N-terminal sequence of the 50 kDa fragment:

5'-GGATA(T/C) GG(T/A) GG(T/A) GC(T/A) GT(T/A) GT(T/A) AT(T/A) GAT CC-3'

23-mer SSDP40K corresponding to the N-terminal sequence of the 40 kDa fragment:

5'-GC(T/A) CC(T/A) GT(T/A) GA(A/G) AA(A/G) AA(A/G) GT-3'.

Each oligonucleotide is labelled at its 5' end with $\chi P^{32}ATP$ by means of T4 polynucleotide kinase and used to screen a genomic library of *S. solfataricus*, strain MT4 (ATCC n. 49155), in the λ gt11vector, at the EcoRI site, according to standard methods. Filter hybridization are made at 45°C with the SSDP40K probe and at 50°C with the SSDP50K probe, in 6 x saline citrate buffer (SSC) as described in Maniatis, T., Fritsch, E. F., and Sambrook, J. (1989) in Molecular Cloning. A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor. Inserts of positive phages pA1, pC5 e pE1 are subcloned into the EcoRI site of the pUC18 vector, and sequenced (Sequenase, USB). The inserts have partial overlapping regions and an open reading frame, as shown in Fig. 1.

Another genomic library obtained in the λ EMBL3 vector with MboI partially digested DNA of *S. solfataricus, producing* fragments of around 15 Kb, according to standard methods. The library is screened with the EcoRI insert of pC5 clone as probe. Hybridizations are performed on filters at 65°C, 6 x SSC, according to Maniatis, T., Fritsch, E. F., and Sambrook, J. (1989) in Molecular Cloning. A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor. Two positive phages λ 4B and λ 2P are purified and digested with restriction enzymes (Fig. 1). The EcoRI-PstI fragment, present in both phages, and able to hybridize with pE1, pA1 and pC5 clones is inserted

into the pEMBL8 vector, producing the plasmid named pFCpolS (DSM N. 7091). The sequence is shown in SEQ ID N1. The sequence shows a region of 882 codons with an open reading frame, in agreement with the 100 kDa molecular weight of the protein. The 5' end non coding region does not comprise promoter sequences homologous to other *Archaea*bacterial promoters (Reiter, W.D., Palm, P., and Zillig, W. (1988) Nucleic Acids Res. 16, 1-19; Reiter, W.D., Hudepohl, U., and Zillig, W. (1990) Proc. Natl. Acad. Sci USA 87, 9509-9513. A pirimidine rich region comprising the TTTTTAT sequence is present at the 3'end of the termination codon, in analogy with other terminators from *Archaea* bacteria (Cubellis, M.V., Rozzo, C., Nitti, G., Arnone, M.I, Marino, G., and Sannia, G. (1989) Eur. J. Biochem. 186, 375-381; Cubellis, M.V., Rozzo, C., Montecucchi, P., and Rossi, M. (1990) Gene 94, 89-94; Reiter, W.D., Palm, P., and Zullig, W. (1989) Nucleic Acid Res. 16, 2445-2459).

Example 3 <u>Sequence homology with other DNA polymerases</u>

A sequence analysis shows homologies with class B DNA polymerases, as viral eucaryote replicases (Gibbs, J.S., Chiou, H.C., Hall, J.D., Mount, D.W., Retondo, M.J., Weller, S.K., and Coen, D.M. (1985) Proc. Natl. Acad. Sci. USA 82, 7969-7973; Kouzarides, T., Bankier, A.T., Satchwell, S.C., Weston, K., Tomlison, P., and Barrel, B.G. (1987) J. Virol. 61, 125-133; Earl, P.L., Jones, E.V., and Moss, B. (1986) Proc. Natl. Acad. Sci. USA 83, 3659-3663), human replicases (Wong, S.W., Wahl, A.F., Yuan, P.M., Arai, N., Pearson, B. E., Arai, K.-I., Korn, D., Hunkapiller, M.W., and Wang, T. S.-F. (1988) EMBO J. 7, 37-47) and DNA polymerase α ofi *S. cerevisiae* (Pizzagalli, A.,

Valsasnini, P., Plevani, P., and Lucchini, G. (1988) Proc. Natl. Acad. Sci. USA 85, 3772-3776). Few homologies are evident with *E. coli* DNA polymerases (Joyce, C.M., Kelley, W.S., and Grindley, N.D. F. (1982) J. Biol. Chem. 257, 1958-1964).

Class B DNA polymerases show conserved motifs (Ito, J., and Braithwaite, D.K. (1991) Nucleic Acids Res. 19, 4045-4057; Wong. S.W., Zahl, A.F., Yuan, P.-M., Arai, N., Pearson, B. E., Arai, K.-I., Korn, D., Hunkapiller, M.W., and Wang, T. S.-F. (1988) EMBO J. 7, 37-47; Iwasaki, H., Ishino, Y., Toh, H., Nakata, A., and Shinagawa, H. (1991) Mol. Gen. Genet. 226, 24-33; Larder, B.A., Kemp, S.D., and Darby, G. (1987) EMBO J. 6, 169-175; Bernard, A., Zaballos, A., Salas,, M., and Blanco, L. (1987) EMBO J. 6, 4219-4225; Blanco, L., Bernard, A., Blasco, M.A., and Salas, M. (1991) Gene 100, 27-38), which are found also in the sequence of the invention, as shown in Figs. 2a and 2b, regions 1-8.

Regions 1, 2 e 3 correspond to EXO motifs found in DNA polymerases with 3'-5' exonuclease activity (Morrison, A., Bell, J.B., Kunkel, T.A., and Sugino, A. (1991) Proc. Natl. Acad. Sci. USA 88, 9473-9477), where three aspartic acid and one glutammic acid residues are maintained.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: Consiglio Nazionale delle Ricerche
(B) STREET: P.le Aldo Moro 5
(C) CITY: Roma
(D) STATE: Italy
(E) COUNTRY: Italy
(F) POSTAL CODE (ZIP): 00185

(ii) TITLE OF INVENTION: Nucleotide sequences coding for a DNA polymerase, DNA polymerase and uses thereof

(iii) NUMBER OF SEQUENCES: 2

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3112 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iii) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Sulfolobus solfataricus

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 198..2846

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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ATCTGGTGTT TTTCTTTCTC ATGCATATTA ATAATGTTTA CTAAGATTCA AGGCATATCT    60

CTTAAGAAAT GGCTAGATGA ATGAGAGGAG CAGGAGTAGC TTAAGAATCT TAAAACTTAG   120

GTTCTTCATA AATGTCTATT TTTTCTCCCG CATTAAAACT TATAGCGTAT TTCTCAGAAA   180

ATAATATATG TTAGAAA ATG ACT AAG CAA CTT ACC TTA TTT GAT ATT CCT      230
                   Met Thr Lys Gln Leu Thr Leu Phe Asp Ile Pro
                    1               5                  10

TCA TCT AAA CCC GCT AAG AGT GAA CAA AAT ACT CAA CAA TCG CAA CAG     278
Ser Ser Lys Pro Ala Lys Ser Glu Gln Asn Thr Gln Gln Ser Gln Gln
            15                  20                  25

AGT GCT CCC GTT GAG GAA AAA AAG GTA GTT AGG AGG GAA TGG CTT GAA     326
Ser Ala Pro Val Glu Glu Lys Lys Val Val Arg Arg Glu Trp Leu Glu
        30                  35                  40

GAG GCT CAG GAA AAT AAG ATA TAC TTC CTA TTG CAA GTA GAT TAT GAT     374
Glu Ala Gln Glu Asn Lys Ile Tyr Phe Leu Leu Gln Val Asp Tyr Asp
    45                  50                  55

GGT AAG AAA GGT AAG GCT GTA TGT AAG CTA TTC GAT AAA GAA ACT CAA     422
Gly Lys Lys Gly Lys Ala Val Cys Lys Leu Phe Asp Lys Glu Thr Gln
60                  65                  70                  75
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AAG ATC TAT GCC CTA TAT GAT AAT ACT GGA CAT AAG CCC TAC TTT CTA     470
Lys Ile Tyr Ala Leu Tyr Asp Asn Thr Gly His Lys Pro Tyr Phe Leu
                 80                  85                  90

GTA GAT CTT GAA CCT GAT AAA GTA GGT AAA ATA CCT AAG ATT GTT AGA     518
Val Asp Leu Glu Pro Asp Lys Val Gly Lys Ile Pro Lys Ile Val Arg
             95                 100                 105

GAT CCA TCT TTT GAT CAC ATA GAG ACT GTG AGT AAG ATA GAC CCG TAT     566
Asp Pro Ser Phe Asp His Ile Glu Thr Val Ser Lys Ile Asp Pro Tyr
        110                 115                 120

ACT TGG AAT AAA TTC AAA TTA ACT AAA ATC GTT GTT AGA GAT CCC CAT     614
Thr Trp Asn Lys Phe Lys Leu Thr Lys Ile Val Val Arg Asp Pro His
    125                 130                 135

GCA GTG AGA AGA TTA AGG AAT GAT GTT CCA AAA GCG TAT GAG GCT CAC     662
Ala Val Arg Arg Leu Arg Asn Asp Val Pro Lys Ala Tyr Glu Ala His
140                 145                 150                 155

ATA AAA TAT TTT AAC AAC TAC ATG TAT GAC ATA GGT CTA ATC CCC GGT     710
Ile Lys Tyr Phe Asn Asn Tyr Met Tyr Asp Ile Gly Leu Ile Pro Gly
                160                 165                 170

ATG CCT TAT GTT GTT AAG AAT GGG AAG TTA GAA AGT GTC TAT TTG TCT     758
Met Pro Tyr Val Val Lys Asn Gly Lys Leu Glu Ser Val Tyr Leu Ser
            175                 180                 185

TTG GAC GAG AAA GAT GTT GAG GAG ATT AAG AAA GCC TTC GCT GAT TCA     806
Leu Asp Glu Lys Asp Val Glu Glu Ile Lys Lys Ala Phe Ala Asp Ser
        190                 195                 200

GAT GAA ATG ACT AGA CAA ATG GCA GTC GAT TGG CTT CCC ATA TTT GAA     854
Asp Glu Met Thr Arg Gln Met Ala Val Asp Trp Leu Pro Ile Phe Glu
    205                 210                 215
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ACT GAA ATA CCT AAA ATA AAA AGG GTT GCG ATA GAT ATT GAG GTA TAT 902
Thr Glu Ile Pro Lys Ile Lys Arg Val Ala Ile Asp Ile Glu Val Tyr
220 225 230 235

ACA CCA GTT AAG GGT AGA ATC CCA GAC TCT CAG AAG GCT GAG TTT CCA 950
Thr Pro Val Lys Gly Arg Ile Pro Asp Ser Gln Lys Ala Glu Phe Pro
240 245 250

ATT ATA AGT ATA GCA TTA GCG GGG AGT GAT GGA TTA AAG AAG GTT CTT 998
Ile Ile Ser Ile Ala Leu Ala Gly Ser Asp Gly Leu Lys Lys Val Leu
255 260 265

GTA TTA AAT AGG AAT GAT GTC AAT GAA GGG AGT GTA AAA CTT GAT GGA 1046
Val Leu Asn Arg Asn Asp Val Asn Glu Gly Ser Val Lys Leu Asp Gly
270 275 280

ATA TCG GTT GAG AGA TTT AAT ACA GAG TAC GAA CTG TTA GGG AGA TTT 1094
Ile Ser Val Glu Arg Phe Asn Thr Glu Tyr Glu Leu Leu Gly Arg Phe
285 290 295

TTT GAT ATA CTG TTA GAA TAT CCG ATA GTT CTT ACA TTC AAT GGA GAC 1142
Phe Asp Ile Leu Leu Glu Tyr Pro Ile Val Leu Thr Phe Asn Gly Asp
300 305 310 315

GAT TTT GAT TTA CCT TAC ATT TAC TTT AGG GCG TTA AAG TTA GGT TAT 1190
Asp Phe Asp Leu Pro Tyr Ile Tyr Phe Arg Ala Leu Lys Leu Gly Tyr
320 325 330

TTT CCA GAG GAA ATT CCC ATA GAT GTA GCT GGT AAG GAT GAA GCC AAG 1238
Phe Pro Glu Glu Ile Pro Ile Asp Val Ala Gly Lys Asp Glu Ala Lys
335 340 345

TAT CTA GCT GGT CTT CAT ATA GAC TTG TAC AAA TTC TTC TTT AAT AAG 1286
Tyr Leu Ala Gly Leu His Ile Asp Leu Tyr Lys Phe Phe Phe Asn Lys
350 355 360

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GCA GTG AGG AAT TAT GCA TTT GAG GGA AAG TAT AAT GAA TAC AAT TTA    1334
Ala Val Arg Asn Tyr Ala Phe Glu Gly Lys Tyr Asn Glu Tyr Asn Leu
    365                 370                 375

GAT GCA GTT GCA AAG GCC TTA TTA GGG ACA TCA AAA GTT AAG GTA GAT    1382
Asp Ala Val Ala Lys Ala Leu Leu Gly Thr Ser Lys Val Lys Val Asp
380                 385                 390                 395

ACG CTA ATA TCT TTC TTA GAT GTA GAA AAA TTA ATA GAA TAT AAC TTT    1430
Thr Leu Ile Ser Phe Leu Asp Val Glu Lys Leu Ile Glu Tyr Asn Phe
                400                 405                 410

AGG GAT GCC GAA ATC ACA CTT CAG CTT ACT ACA TTT AAT AAC GAC CTA    1478
Arg Asp Ala Glu Ile Thr Leu Gln Leu Thr Thr Phe Asn Asn Asp Leu
            415                 420                 425

ACT ATG AAG TTA ATT GTA TTG TTT TCT AGA ATT TCT AGA CTA GGA ATT    1526
Thr Met Lys Leu Ile Val Leu Phe Ser Arg Ile Ser Arg Leu Gly Ile
        430                 435                 440

GAG GAA TTA ACT CGG ACA GAA ATA TCT ACT TGG GTA AAG AAT TTA TAT    1574
Glu Glu Leu Thr Arg Thr Glu Ile Ser Thr Trp Val Lys Asn Leu Tyr
    445                 450                 455

TAT TGG GAA CAT AGA AAA AGA AAT TGG TTA ATT CCT CTT AAG GAA GAA    1622
Tyr Trp Glu His Arg Lys Arg Asn Trp Leu Ile Pro Leu Lys Glu Glu
460                 465                 470                 475

ATC TTA GCG AAA TCC TCT AAT ATA AGA ACT TCT GCT CTA ATA AAG GGA    1670
Ile Leu Ala Lys Ser Ser Asn Ile Arg Thr Ser Ala Leu Ile Lys Gly
                480                 485                 490

AAA GGA TAT AAA GGC GCA GTA GTT ATA GAC CCA CCT GCT GGA ATA TTC    1718
Lys Gly Tyr Lys Gly Ala Val Val Ile Asp Pro Pro Ala Gly Ile Phe
            495                 500                 505
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TTT AAC ATA ACT GTT TTA GAT TTT GCA TCA CTA TAT CCT TCA ATA ATT 1766
Phe Asn Ile Thr Val Leu Asp Phe Ala Ser Leu Tyr Pro Ser Ile Ile
510 515 520

AGA ACG TGG AAT CTT AGT TAC GAG ACT GTA GAC ATT CAA CAA TGT AAG 1814
Arg Thr Trp Asn Leu Ser Tyr Glu Thr Val Asp Ile Gln Gln Cys Lys
525 530 535

AAG CCC TAT GAA GTA AAG GAT GAG ACA GGG GAG GTG CTA CAT ATA GTT 1862
Lys Pro Tyr Glu Val Lys Asp Glu Thr Gly Glu Val Leu His Ile Val
540 545 550 555

TGC ATG GAT AGG CCA GGT ATA ACA GCA GTA ATA ACT GGG TTA CTA AGA 1910
Cys Met Asp Arg Pro Gly Ile Thr Ala Val Ile Thr Gly Leu Leu Arg
560 565 570

GAC TTC AGA GTA AAG ATA TAC AAA AAG AAA GCG AAG AAC CCT AAT AAT 1958
Asp Phe Arg Val Lys Ile Tyr Lys Lys Lys Ala Lys Asn Pro Asn Asn
575 580 585

AGT GAG GAA CAA AAA CTA CTC TAT GAC GTA GTA CAG AGA GCA ATG AAA 2006
Ser Glu Glu Gln Lys Leu Leu Tyr Asp Val Val Gln Arg Ala Met Lys
590 595 600

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605 610 615

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620 625 630 635

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Val Ile Thr Ser Thr Val Lys Lys Ala Arg Glu Glu Gly Leu Thr Val
640 645 650

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Leu Tyr Gly Asp Thr Asp Ser Leu Phe Leu Leu Asn Pro Pro Lys Asn
655 660 665

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Ser Leu Glu Asn Ile Ile Lys Trp Val Lys Thr Thr Phe Asn Leu Asp
670 675 680

TTG GAA GTT GAT AAA ACC TAC AAG TTT GTG GCT TTT TCT GGA TTG AAG 2294
Leu Glu Val Asp Lys Thr Tyr Lys Phe Val Ala Phe Ser Gly Leu Lys
685 690 695

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700 705 710 715

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720 725 730

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735 740 745

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750 755 760

AAA CTA AAA AAC AAA GGA TAC AAT CTG GAC GAA TTA GCG TTT AAA GTA 2534
Lys Leu Lys Asn Lys Gly Tyr Asn Leu Asp Glu Leu Ala Phe Lys Val
765 770 775

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780 785 790 795

GTA AAG GCA GCT CTA CAA CTT AGA CCA TTT GGA GTT AAC GTA TTA CCA 2630
Val Lys Ala Ala Leu Gln Leu Arg Pro Phe Gly Val Asn Val Leu Pro
800 805 810

CGA GAT ATA ATA TAC TAT GTT AAG GTT AGA TCT AAA GAT GGA GTG AAA 2678
Arg Asp Ile Ile Tyr Tyr Val Lys Val Arg Ser Lys Asp Gly Val Lys
815 820 825

CCA GTA CAA CTA GCT AAA GTT ACT GAA ATA GAC GCA GAG AAA TAT TTA 2726
Pro Val Gln Leu Ala Lys Val Thr Glu Ile Asp Ala Glu Lys Tyr Leu
830 835 840

GAA GCG TTA AGA AGT ACG TTT GAG CAA ATC TTA AGG GCA TTC GGA GTC 2774
Glu Ala Leu Arg Ser Thr Phe Glu Gln Ile Leu Arg Ala Phe Gly Val
845 850 855

TCT TGG GAT GAG ATA GCA GCC ACA ATG TCG ATA GAT TCG TTC TTT TCA 2822
Ser Trp Asp Glu Ile Ala Ala Thr Met Ser Ile Asp Ser Phe Phe Ser
860 865 870 875

TAC CCA AGT AAA GGA AAT AGT TAATTAAGAA AGATAGCAAT TCTTCATAAT 2873
Tyr Pro Ser Lys Gly Asn Ser
880

AAATTTTTAG AAGCAATTTT TACCCACATA AGTTATAAAG ATTTTTAGAA AATTTAAATC 2933

GTATATTTTT ATTCTTCCTC CTCTTCCTCT AATTCTTCCT TTAATTCTTC TTGTTTCTGC 2993

ATACCCAAGT AAAGGAAATA GTTAATTAAG AAAGATAGCA ATTCTTCATA ATAAATTTTT 3053

AGAAGCAATT TTTACCCACA TAAGTTATAA AGATTTTTAG AAAATTTAAA TCGTATATT 3112

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 882 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

```
Met Thr Lys Gln Leu Thr Leu Phe Asp Ile Pro Ser Ser Lys Pro Ala
 1               5                  10                  15

Lys Ser Glu Gln Asn Thr Gln Gln Ser Gln Gln Ser Ala Pro Val Glu
            20                  25                  30

Glu Lys Lys Val Val Arg Arg Glu Trp Leu Glu Glu Ala Gln Glu Asn
        35                  40                  45

Lys Ile Tyr Phe Leu Leu Gln Val Asp Tyr Asp Gly Lys Lys Gly Lys
    50                  55                  60

Ala Val Cys Lys Leu Phe Asp Lys Glu Thr Gln Lys Ile Tyr Ala Leu
65                  70                  75                  80

Tyr Asp Asn Thr Gly His Lys Pro Tyr Phe Leu Val Asp Leu Glu Pro
                85                  90                  95

Asp Lys Val Gly Lys Ile Pro Lys Ile Val Arg Asp Pro Ser Phe Asp
            100                 105                 110

His Ile Glu Thr Val Ser Lys Ile Asp Pro Tyr Thr Trp Asn Lys Phe
        115                 120                 125
```

Lys Leu Thr Lys Ile Val Val Arg Asp Pro His Ala Val Arg Arg Leu
130 135 140

Arg Asn Asp Val Pro Lys Ala Tyr Glu Ala His Ile Lys Tyr Phe Asn
145 150 155 160

Asn Tyr Met Tyr Asp Ile Gly Leu Ile Pro Gly Met Pro Tyr Val Val
165 170 175

Lys Asn Gly Lys Leu Glu Ser Val Tyr Leu Ser Leu Asp Glu Lys Asp
180 185 190

Val Glu Glu Ile Lys Lys Ala Phe Ala Asp Ser Asp Glu Met Thr Arg
195 200 205

Gln Met Ala Val Asp Trp Leu Pro Ile Phe Glu Thr Glu Ile Pro Lys
210 215 220

Ile Lys Arg Val Ala Ile Asp Ile Glu Val Tyr Thr Pro Val Lys Gly
225 230 235 240

Arg Ile Pro Asp Ser Gln Lys Ala Glu Phe Pro Ile Ile Ser Ile Ala
245 250 255

Leu Ala Gly Ser Asp Gly Leu Lys Lys Val Leu Val Leu Asn Arg Asn
260 265 270

Asp Val Asn Glu Gly Ser Val Lys Leu Asp Gly Ile Ser Val Glu Arg
275 280 285

Phe Asn Thr Glu Tyr Glu Leu Leu Gly Arg Phe Phe Asp Ile Leu Leu
290 295 300

Glu Tyr Pro Ile Val Leu Thr Phe Asn Gly Asp Asp Phe Asp Leu Pro
305 310 315 320

```
Tyr Ile Tyr Phe Arg Ala Leu Lys Leu Gly Tyr Phe Pro Glu Glu Ile
                325                 330                 335

Pro Ile Asp Val Ala Gly Lys Asp Glu Ala Lys Tyr Leu Ala Gly Leu
            340                 345                 350

His Ile Asp Leu Tyr Lys Phe Phe Phe Asn Lys Ala Val Arg Asn Tyr
        355                 360                 365

Ala Phe Glu Gly Lys Tyr Asn Glu Tyr Asn Leu Asp Ala Val Ala Lys
    370                 375                 380

Ala Leu Leu Gly Thr Ser Lys Val Lys Val Asp Thr Leu Ile Ser Phe
385                 390                 395                 400

Leu Asp Val Glu Lys Leu Ile Glu Tyr Asn Phe Arg Asp Ala Glu Ile
                405                 410                 415

Thr Leu Gln Leu Thr Thr Phe Asn Asn Asp Leu Thr Met Lys Leu Ile
            420                 425                 430

Val Leu Phe Ser Arg Ile Ser Arg Leu Gly Ile Glu Glu Leu Thr Arg
        435                 440                 445

Thr Glu Ile Ser Thr Trp Val Lys Asn Leu Tyr Tyr Trp Glu His Arg
    450                 455                 460

Lys Arg Asn Trp Leu Ile Pro Leu Lys Glu Glu Ile Leu Ala Lys Ser
465                 470                 475                 480

Ser Asn Ile Arg Thr Ser Ala Leu Ile Lys Gly Lys Gly Tyr Lys Gly
                485                 490                 495

Ala Val Val Ile Asp Pro Pro Ala Gly Ile Phe Phe Asn Ile Thr Val
            500                 505                 510
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Leu Asp Phe Ala Ser Leu Tyr Pro Ser Ile Ile Arg Thr Trp Asn Leu
        515                 520                 525

Ser Tyr Glu Thr Val Asp Ile Gln Gln Cys Lys Lys Pro Tyr Glu Val
    530                 535                 540

Lys Asp Glu Thr Gly Glu Val Leu His Ile Val Cys Met Asp Arg Pro
545                 550                 555                 560

Gly Ile Thr Ala Val Ile Thr Gly Leu Leu Arg Asp Phe Arg Val Lys
                565                 570                 575

Ile Tyr Lys Lys Lys Ala Lys Asn Pro Asn Asn Ser Glu Glu Gln Lys
            580                 585                 590

Leu Leu Tyr Asp Val Val Gln Arg Ala Met Lys Val Phe Ile Asn Ala
        595                 600                 605

Thr Tyr Gly Val Phe Gly Ala Glu Thr Phe Pro Leu Tyr Ala Pro Arg
    610                 615                 620

Val Ala Glu Ser Val Thr Ala Leu Gly Arg Tyr Val Ile Thr Ser Thr
625                 630                 635                 640

Val Lys Lys Ala Arg Glu Glu Gly Leu Thr Val Leu Tyr Gly Asp Thr
                645                 650                 655

Asp Ser Leu Phe Leu Leu Asn Pro Pro Lys Asn Ser Leu Glu Asn Ile
            660                 665                 670

Ile Lys Trp Val Lys Thr Thr Phe Asn Leu Asp Leu Glu Val Asp Lys
        675                 680                 685

Thr Tyr Lys Phe Val Ala Phe Ser Gly Leu Lys Lys Asn Tyr Phe Gly
    690                 695                 700
```

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Val Tyr Gln Asp Gly Lys Val Asp Ile Lys Gly Met Leu Val Lys Lys
705                 710                 715                 720

Arg Asn Thr Pro Glu Phe Val Lys Lys Val Phe Asn Glu Val Lys Glu
                725                 730                 735

Leu Met Ile Ser Ile Asn Ser Pro Asn Asp Val Lys Glu Ile Lys Arg
            740                 745                 750

Lys Ile Val Asp Val Val Lys Gly Ser Tyr Glu Lys Leu Lys Asn Lys
        755                 760                 765

Gly Tyr Asn Leu Asp Glu Leu Ala Phe Lys Val Met Leu Ser Lys Pro
    770                 775                 780

Leu Asp Ala Tyr Lys Lys Asn Thr Pro Gln His Val Lys Ala Ala Leu
785                 790                 795                 800

Gln Leu Arg Pro Phe Gly Val Asn Val Leu Pro Arg Asp Ile Ile Tyr
                805                 810                 815

Tyr Val Lys Val Arg Ser Lys Asp Gly Val Lys Pro Val Gln Leu Ala
            820                 825                 830

Lys Val Thr Glu Ile Asp Ala Glu Lys Tyr Leu Glu Ala Leu Arg Ser
        835                 840                 845

Thr Phe Glu Gln Ile Leu Arg Ala Phe Gly Val Ser Trp Asp Glu Ile
    850                 855                 860

Ala Ala Thr Met Ser Ile Asp Ser Phe Phe Ser Tyr Pro Ser Lys Gly
865                 870                 875                 880

Asn Ser
```

CLAIMS

1. Nucleic acid of natural, recombinant or synthetic origin, comprising a nucleotide sequence coding a polypeptide or fragments thereof having a thermostable and thermofilic DNA polymerase activity.

2. Nucleic acid according to Claim 1 wherein said nucleotide sequence is derived from bacteria of the *Archaea*domain.

3. Nucleic acid according to Claim 2 wherein said nucleotide sequence is derived from bacteria of the *Sulfolobus* genus.

4. Nucleic acid according to Claim 2 wherein said nucleotide sequence is derived from bacteria of the *S. solfataricus*species.

5. Nucleic acid according to any of previous Claims wherein said polypeptide or fragments thereof have also a 3'-5' exonuclease activity.

6. Nucleic acid according to Claim 5 wherein said nucleotide sequence codes the polypeptide having the aminoacid sequence of SEQ ID N2 or fragments thereof.

7. Nucleic acid according to Claim 6 wherein said nucleotide sequence codes the polypeptide having the aminoacid sequence of SEQ ID N2 or fragments thereof, deleted or substituted for one or more aminoacids, so that said DNA polymerase activity is maintained.

8. Nucleic acid comprised in the sequence of SEQ ID N1 characterized in that from nucleotide 1 to nucleotide 197 is a non coding sequence, from nucleotide 198 to nucleotide 2843 coding a polypeptide with a thermostable and thermofilic DNA polymerase activity and from nucleotide 2844 to nucleotide 3112 is a non coding sequence.

9. Nucleic acid according to Claim 8 wherein said coding sequence lacks or is substituted of one or more nucleotides so that said DNA polymerase activity is maintained.

10. Nucleic acid able to hybridize at least at medium stringency to a nucleic acid according to any of previous Claims.

11. Nucleic acid according to Claim 10 complementary to nucleotide sequences from Claim 1 to 9.

12. Polypeptide with a thermostable and thermofilic DNA polymerase activity.

13. Polypeptide according to Claim 12 produced through recombinant DNA techniques by nucleic acids according to any of previous Claims from 1 to 11.

14. Polypeptide according to Claim 13 produced by the nucleotide sequence comprised in SEQ ID N1.

15. Polypeptide according to Claim 14 having a sequence comprised in SEQ ID N2.

16. Recombinant cloning or expression vectors, having a plasmid or viral derivation, comprising nucleotide sequences accoprding to any of previous Claims from 1 to 11.

17. Recombinant vector according to Claim 16 being the plasmid pFCpolS (DSM N.7091).

18. Cells transformed with vectors according to Claims 16 or 17.

FIG. 1

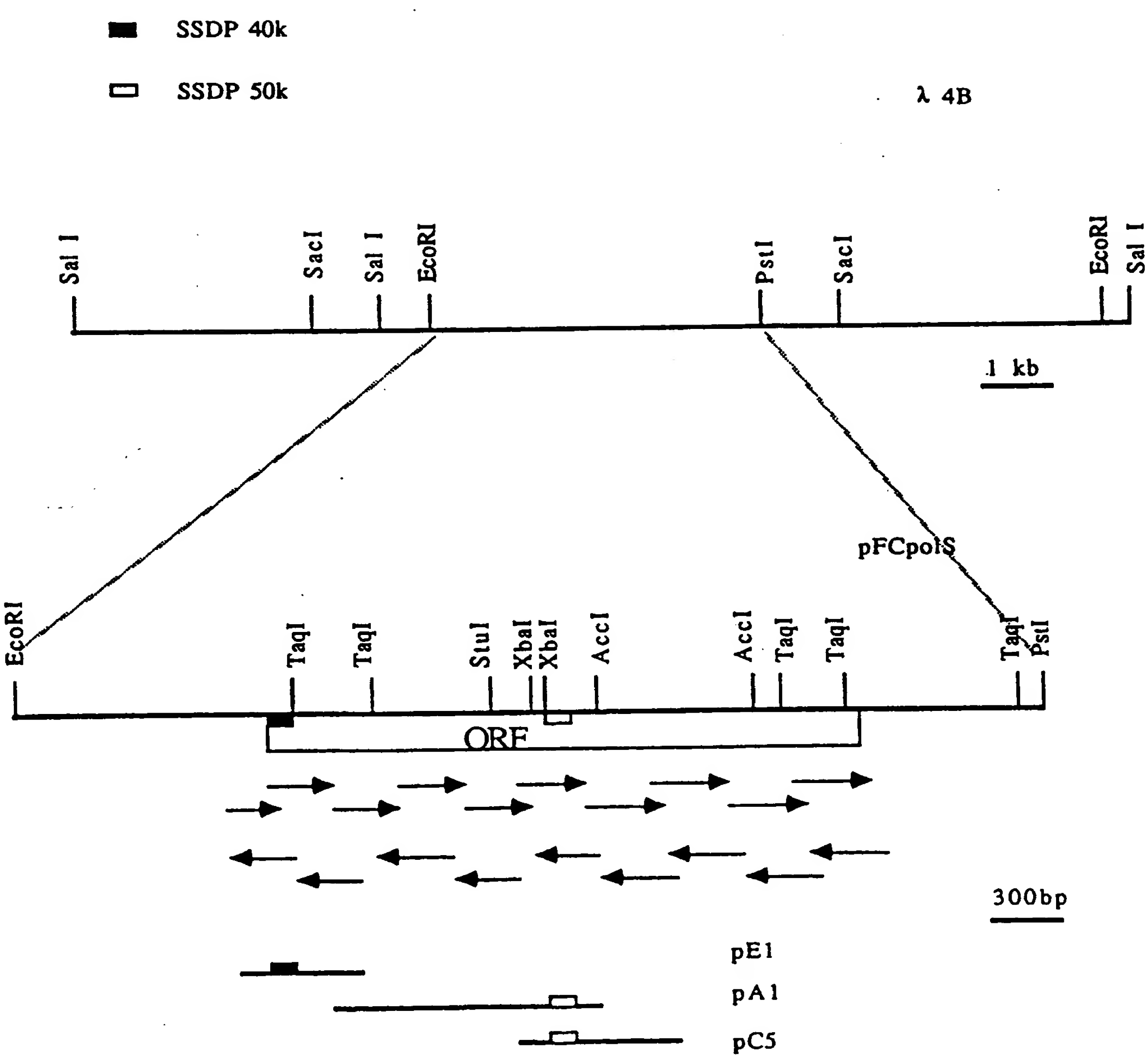
SSDP 40k
SSDP 50k
λ 4B
Sal I
SacI
Sal I
EcoRI
PstI
SacI
EcoRI
Sal I
1 kb
pFCpolS
EcoRI
TaqI
TaqI
StuI
XbaI
XbaI
AccI
AccI
TaqI
TaqI
TaqI
PstI
ORF
300bp
pE1
pA1
pC5

FIG. 2a

```
REGION 1                     REGION 2                        REGION 3
Sso     227  RVAIDIEVYT      309  VLTFMGDDFDLPYIYFR          406  LIEYNFRDAEI
HSV     364  LMCFDIECKA      462  VTGYNIINFDWPFLLAK          574  IGEYCIQDSLL
ScII    286  VMAFDIETTK      374  ISTFNGDFFDWPFINNR          470  LSEYSVSDAVA
Ade02   137  FVTYDVETYT      271  IVGHNINGFDEIVLAAQ          435  TLDYCALDVQV
VacV    137  YLFLDIECHF      234  VVTFNGHNFDLRYITNR          366  TGNYVTVDEDI
T4      108  VANCDIEVTG      210  FTGWNIEGFDVPYIMNR          366  YISYNIIDVES
φ29       8  MYSCDFETTT       58  LYFHNL-KFDGAFIINW          162  EYAYIKNDIQI
EcIIIε    8  QIVLDTETTG       95  LVIHNA-AFDIGFMDYE          149  CARY-SIDNSK
T7        1  MIVSDIEANA       56  IVFHNGHKYDVPALTKL          167  MNDYNVQDVVV
EcI     351  YFAFDTETDS      416  KVGQNL-KYDRGILANY          494  AGRYAAEDADV
                 * *                    *                              *

REGION 4     •                                    •   •
Sso     494  YKGAVVIDPP--AGIFFN-ITVLDFASLYPSIIRTWNLSYET-V
HSV     696  YQGAKVLDPT--SGFHVNPVVVFDFASLYPSIIQAHNLCFST-L
Ade02   522  IRGGRCYPTY--LGILREPLYVYDICGMYASAL-THPMPWGPPL
VacV    503  YEGGKVFAPKQ-KMFSNN-VLIFDYMSLYPNVCIFGNLSPET-L
Hsα     839  YAGGLVLDPK--VGFYDKFILLLDFNSLYPSIIQEFNICFTT-V
ScI     843  YQGGLVFEPE--KGLHKNYVLVMDFNSLYPSIIQEFNICFTT-V
EcII    398  SPGGYVMDSR--PGLYD-SVLVLDYKSLYPSIIRTFLIDPVG-L
T4      388  FPGAFVFEPK---PIARRYIMSFDLTSLYPSIIRQVNISPETIR
φ29     226  YRGGFTWLNDRFKEKEIGEGMVFDVNSLYPAQMYSRLLPYGEPI
```

```
REGION 5
                                          •                                 •   •
Sso     594   L Y D V V Q R A M K V F I N A T Y G V F G A E T F P L Y A P R V A E S V T A L G - R
HSV     802   L L D K Q Q A A I K V V C N S V Y G F T G V Q H G L L P C L H V A A T V T T I G - R
Ade02   686   K N Q T L R S I A K L L S N A L Y G S F A T K L D N K K - I V F S D Q M D A A T L K
VacV    627   I Y D S M Q Y T Y K I V A N S V Y G L M G F R N S A L Y S Y A S A K S C T S I G - R
Hsα     941   Q Y D I R Q K A L K L T A N S M Y G C L G F S Y S R F Y A K P L A A L V T Y K G - R
ScI     935   Q C D I R Q Q A L K L T A N S M Y G C L G Y V N S R F Y A K P L A M L V T N K G - R
EcII    483   G N K P L S Q A L K I I M N A F Y G V L G T T A C R F F D P R L A S S I T M R G - H
T4      548   L A M T N Q L N R K I L I N S L Y G A L G N I H F R Y Y D L R N A T A I T I F G Q V
φ29     374   S E G A I K Q L A K L M L N S L Y G K F A S N P D V T G K V P Y L K E N G A L G F R
```

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REGION 6                                                      REGION 7
Sso     648   G L T V L Y G D T D S L F L L N P P K N S L E    698   L K K N Y F G V Y
HSV     879   S M R I I Y G D T D S I F V L C R G L T A A G    937   A K K K Y I G V I
Ade02   863   P L K S V Y G D T D S L F V T E R G H R L M E    934   A P K L Y A - L K
VacV    719   R F R S V Y G D T D S V F T E I D S Q D V D K    777   S K K K Y T T M K
Hsα     995   N L E V I Y G D T D S I M I N T N S T N L E E   1051   K K K K Y A A L V
ScI     989   N L L V V Y G D T D S V M I D T G C D N Y A D   1045   A K K K Y A A L T
EcII    537   G Y D V I Y G D T D S T F V W L K G A H S E E    612   S K K R Y A G L I
T4      612   D F - I A A G D T D S V Y V C V D K - - V I E    701   A K K R Y A - L N
φ29     449   Y D R I I Y C D T D S I H L T G T E I P D V -    490   K R A K Y - L R Q
```

```
REGION 8
Sso     710   K V D - I K G M - L V K K R N T P E
HSV     949   K M L - I K G V D L V R K N N C A F
Ade02   955   K - L R A K G H A A - E G L D Y D T
VacV    799   R - I - N K G T S E T R R D V S K F
Hs     1071   K Q E - L K G L D I V R R D W C D L
ScI    1965   V L E - V K G L D M K R R E F C P L
EcII    627   R M V - F K G L E T V R T D W T P L
T4      721   H - L K I M G M E T - Q Q S S T P K
φ29     512   K - L V E - G S P D - D Y T D I K F
```

FIG. 2b

INTERNATIONAL SEARCH REPORT

International Application No PCT/IT 93/00058

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 C12N15/54; C12N15/63; C12N9/12

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	C12N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X	EMBL Database Accesion number X64466; 30 May 1992 ---	1-6,8, 12-15
X	EP,A,0 455 430 (NEW ENGLAND BIOLABS, INC) 6 November 1991 see page 4, line 3 - line 16; examples I-VI ---	1,2,5, 12-16,18
X	THE ITALIAN JOURNAL OF BIOCHEMISTRY vol. 39, no. 2, April 1990, pages 83 - 99 R. RELLA ET AL. 'Purification and properties of a thermophilic and thermostable DNA polymerase from the Archaebacterium Sulfolobus solfataricus' see page 83, paragraph 3 - page 84, paragraph 1 see page 94, paragraph 1 -paragraph 3; figures 7,8 ---	12-15

-/--

° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

4

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
13 SEPTEMBER 1993	23. 09. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	MONTERO LOPEZ B.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	SYSTEM. APPL. MICROBIOL. vol. 7, 1986, pages 337 - 341 M. ROSSI ET AL. 'Struture and properties of a thermophilic and thermostable DNA polymerase isolated from Sulfolobus solfataricus' see abstract see page 340, left column, paragraph 2; figure 4 ---	12-15
P,X	NUCLEIC ACIDS RESEARCH. vol. 20, no. 11, 11 June 1992, ARLINGTON, VIRGINIA US pages 2711 - 2716 PISANI, F.M. ET AL. 'A DNA polymerase from the archaeon Sulfolobus solfataricus shows sequence similarity to family B DNA polymerases' -----	1-6,8, 12-18

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

IT 9300058
SA 75960

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 13/09/93

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A-0455430	06-11-91	US-A- 5210036	11-05-93
		JP-A- 5068547	23-03-93

EPO FORM P0479

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82